

inosinic acid derivatives;

(5) the hybridization probe of (1), wherein the added nucleotide sequence per se is incapable of hybridizing to any nucleotide sequences under stringent hybridization conditions for the DNA to be labeled;

(6) a method for detecting, with the hybridization probe of any one of (1) to (5), a nucleic acid having a nucleotide sequence complementary to the DNA to be labeled;

(7) the method of (6), wherein RNA or cDNA library is detected;

(8) a method for labeling a DNA by 3'-tailing with terminal transferase, wherein nucleotides and/or nucleotide derivatives having weaker affinity of hydrogen bonding in base pairing when compared with those of hydrogen bonding in an a/t pair, in an a/u pair, and in a g/c pair and which can be substrates in nucleotide-adding reaction with terminal transferase are used as substrates;

(9) the method of (8), wherein the nucleotide is deoxyinosine 5'-triphosphate;

(10) the method of (8), wherein the nucleotides and/or nucleotide derivatives having weaker affinity in base pairing are mixed with labeled nucleotides or nucleotide derivatives and used as the substrates;

(11) a kit for synthesizing a hybridization probe, the kit comprising

i) nucleotides and/or nucleotide derivatives

(a) having weaker affinity of hydrogen bonding in base pairing with bases of the target nucleotide sequence when compared with those of hydrogen bonding in an a/t pair, in an a/u pair, and in a g/c pair; and

(b) being introduced into a DNA to be labeled through nucleotide-adding reaction with terminal transferase;

ii) labeled nucleotides or nucleotide derivatives; and

iii) terminal transferase; and

(12) a method for preventing hybridization of a hybridization probe in which a nucleotide sequence comprising labeled nucleotides is added to a DNA to be labeled, the hybridization non-specific to

the sequence of the DNA to be labeled, wherein the nucleotides and/or nucleotide derivatives having weaker affinity of hydrogen bonding in base pairing when compared with those of hydrogen bonding in an a/t pair, in an a/u pair, and in a g/c pair are inserted into the added nucleotide sequence.

The "DNA to be labeled" used herein means a DNA capable of specifically binding with a target nucleotide sequence through hybridization. In general, such a DNA is designated as "probe". The DNA typically comprises a nucleotide sequence complementary to the target nucleotide sequence. Alternatively, the DNA can be designed to be able to hybridize with the target when having some mutated nucleotides. Such a DNA can hybridize to the target nucleotide sequence under stringent conditions, and is long enough to stably maintain the formed duplex without being dissociated even when subjected to washing treatment under typical conditions. This DNA to be labeled can also be used without any special modification as has been used previously. Specifically, for each purpose of detection, the DNA may be a chemically synthesized oligonucleotide or DNA fragment resulting from digestion of plasmid or chromosome. Alternatively, the DNA can be PCR products or cDNA prepared by enzymatic nucleotide synthesis, or a fragment thereof. On the other hand, the target nucleotide sequence can be a DNA, RNA, DNA-RNA hybrid, or the like, and there is no special restriction on the type of nucleotide sequence as long as base pairing can occur with the target nucleotide sequence. The specificity and affinity in the hybridization between the target nucleotide sequence and the probe depend on the type of base responsible for base pairing and on the reaction conditions. Determinants having an impact on hybridization and typical hybridization conditions are summarized below (Molecular Cloning, Cold spring harbor laboratory press, 1989).

Temperature of the reaction solution	68°C
Salt concentration	6x SSC
(20x SSC: 3 M NaCl, 0.3 M sodium citrate)	
pH	7.0
Sodium dodecyl sulfate	0.5%

5x Denhardt's reagent

(composition of 50x Denhardt's reagent:

0.01 g/mL Ficoll type 400, Pharmacia;

0.01 g/mL polyvinyl pyrrolidone;

5 0.01 g/mL bovine serum albumin Factor V, Shigma)

In the present invention, the hybridization probe is provided by adding a nucleotide sequence comprising labeled nucleotides or nucleotide derivatives to the DNA to be labeled. The nucleotide
10 sequence to be added comprises labeled nucleotides or nucleotide derivatives and has the following features, (a) and (b):

(a) comprising nucleotides and/or nucleotide derivatives having weaker affinity of hydrogen bonding in base pairing with bases of the target nucleotide sequence when compared with those of hydrogen
15 bonding in an a/t pair, in an a/u pair, and in a g/c pair; and

(b) being able to be introduced into the DNA to be labeled through nucleotide-adding reaction with terminal transferase.

The term "nucleotides having weaker affinity of hydrogen bonding in base pairing when compared with those of hydrogen bonding
20 in an a/t pair, in an a/u pair, and a g/c pair" means nucleotides having weaker affinity for all the bases of a, t, g, c, and u than affinity of the respective general complementary bases, t, a, c, g, and a. Alternatively, it can be defined by the expression that the nucleotides have only weaker affinity for any of the bases contained
25 in the target nucleotide sequence than the affinity of the typical partners in complementary base pairing. Further, the term "nucleotide derivatives" means compounds obtained by introducing a functional group into a nucleotide. Further, chemically synthesized nucleotides mimicking the structure of a naturally occurring
30 nucleotide, and nucleotides provided by chemically modifying a naturally occurring nucleotide to reduce the affinity thereof in base pairing are also included in the nucleotide derivatives exhibiting weak affinity in base pairing in accordance with the present invention, when meeting the above-mentioned requirements, (a) and (b).

35 The method of the present invention is characterized by the nucleotides, nucleotide derivatives, and nucleotide compounds that